The laser scanner is a reliable method to estimate the biomass of a Senegalese sole (*Solea senegalensis*) population in a tank

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Abstract

The measurement of total fish biomass is an essential practice in the aquaculture management. The method commonly used which involves removing a sub-sample of fish from a tank, weighing it and extrapolating the result to the whole tank, carries a large error, is intense labor and causes great stress. Here, we tested a laser scanning method to estimate the total fish biomass from the total fish volume of a sole population (*Solea senegalensis*) in a tank. The ratio FB/FLV of Fish Biomass (FB), weighing the 100 % of soles, versus the Fish layer volume (FLV) measured by the laser scanning, is calculated. Different fish size (small and large) and stocking densities (very low, low, medium and high) were tested. To test the method in the worst conditions, in Very Low stocking density, fish were 3.0 g ± 1.1 (individual mean weight ± SD); but in Low, Medium and High stocking density fish were 234.0 g ± 84.6 (individual mean weight ± SD). The fish layer volume included the fish biomass and the interstitial water present among them, which can be estimated from the ratio FB/FLV. In Medium and High rearing densities with larger fish the ratio takes values very close to 1 (0.957 ± 0.021 and 0.967 ± 0.011) giving percentages of interstitial water lower than 5%. But in Very Low stocking density (0.4 kg/m2) with smaller fish (3.0 g ± 1.1), the ratio FB/FLV was much lower, giving a non-realistic percentage of interstitial water estimation. The low ratios obtained at very low stocking densities are due to the resolution of the image catching

process, which is aggravated when working with small fish, since the error of a pixel from a digital image represents a larger percentage of error than with larger fish and higher stocking density. It should be noted that the Coefficient of Variation (CV) obtained was very low (in all cases lower than 7.2 %) and decreased as the stocking density increased achieving the lowest value (1.1 %) at High stocking density. The laser scanning has proven to be a useful tool to estimate the total fish layer volume of flatfish, and thus fish biomass, in an aquaculture tank with a usual grow-out stocking density for sole, reducing the labor involved and the stress commonly associated to manual sampling.

Keywords: Laser scanning, Biomass estimation, Flatfish

1. Introduction

One of the most common and important practice in the aquaculture management is the measure of total fish biomass. It is important to enable effective management of feeding regimes, oxygen consumption calculation, antibiotic dose, grading times and the optimum time of harvest. Nowadays, in aquaculture facilities, the most common way to estimate total fish biomass is by removing a sub-sample of fish from a tank, weighing it and extrapolating the result to the whole tank. However, it is labor intense and a human action is necessary on the fish or on the tank. Furthermore, an inaccuracy of 15 - 25 % is inherent in this method (Klontz, 1993), the value may vary depending on many factors like fish and sample size as well as frequency of sampling and even the fish species can affect the results. Finally, any operation which involves disturbing and handling fish, like weighing fish manually, can cause physical damage or stress that is a major factor in the growth and health of farmed fish (Ashley, 2007).

The possibility to measure the fish in a tank without human intervention is therefore of great interest for the aquaculture community. This has lead to the emergence of other innovative technologies such as acoustic methods (Løvik, 1987; Conti *et al.*, 2006), model-based methods (Alver *et al.*, 2005), or, the most remarkable, computer vision techniques (Zion, 2012). Computer-imaging techniques in aquaculture are widely used to monitor behavior and welfare of fish (Cordero *et al.*, 1994; Kato *et al*., 1996; Conte, 2004; Kristiansen *et al*., 2004; Stien *et al.*, 2007; Duarte *et al*., 2009), to counting fish (Costa *et al*., 2009), to stock identification (Cadrin and Friedland, 1999; White *et al.*, 2006;), and to estimate size and weight (Ruff *et al.*, 1995; Bedow *et al.*, 1996; Sheih and Petrell, 1998; Tillet *et al*., 2000; Lines *et al.*, 2001; Martinez-de Dios *et al.*, 2003; Costa *et al.*, 2006, 2009; Hufschmied *et al.*, 2011).

Most of the techniques that use the stereo-vision methodology consist in two views of a fish to estimate the fish dimensions in 3-D (Ruff *et al.*, 1995; Bedow *et al*., 1996; Tillet *et al.*, 2000; Lines *et al.*, 2001; Martinez-de Dios *et al.*, 2003; Costa *et al.*, 2006, 2009). Based on this

methodology, AQ1 Systems developed commercially the *AQ1 AM100* to measure and count fish in cages (AQ1 Systems, 2013). The main advantage of these methods is that they are able to measure the fish remotely avoiding the stress caused by the sampling handling. Otherwise the usual conditions in commercial facilities can limit their use because limited visibility, variations in lighting, varying distances and relative orientations between cameras and fish, and motion and density of the monitored fish. Another methodology used nowadays is the commercial development by *Vaki Aquaculture Systems Ltd*. (VAKI, 2009) that provides good dimensional information, although only a single fish can be analyzed and it has to swim through a frame.

Although these methods have proven effective and non-intrusive, they have been used mainly with pelagic fish. These techniques are difficult to implement in flatfish facilities due to its behavior and morphological characteristics. They are relatively inactive species that remain most of the time motionless in the bottom of the tank, therefore they use mainly the surface area instead of the water column and usually they are kept at densities higher than 100 % of coverage area. Taking advantage of the relative immobility and benthic behavior of flatfishes, Oca *et al.* (2007) proposed a laser scanning method based on image analysis. Laser scanning is a prosperous data acquisition method with rapid development since the mid 1990s because it allows an automated sampling of the object surface within a short time (Pfeifer and Briese 2007). Structured light or laser scanning involves projecting a pattern of light onto the target acquiring a multitude of XY or XYZ coordinates (2D or 3D analysis, respectively) from the surface of the object. The laser scanning technique is used in a wide variety of industries, among others, in manufacturing, aerospace and electronic industries. Also in the healthcare sector, for instance, to manufacture prosthesis. In security industries, it's used in some airports for face recognition-based systems (Bogue 2010), and increasingly, it is used in agriculture (Rosell *et al.*, 2009; Igathinathane *et al.*, 2010). In fisheries, Storbeck and Daan (1991) also used

satisfactorily (± 5% of error) the laser light combined with image analysis, to estimate individually weight of dead flatfish to sort them on board of the ship before they were stored.

Almansa *et al.* (2012), used the laser scanning method to evaluate turbot distribution in a raceway tank under different water conditions in a commercial facility with high stocking density (around 30 Kg m^{-2}). In that work, the possibility to use the same methodology to estimate the fish volume of a turbot (*Scophthalmus maximus*) population with a low coefficient of variation (lower than 10%) in a non-invasive way was pointed out. The volume of fish was converted into total biomass assuming that fish density was equal to water density.

The aim of the present work is to validate the feasibility of the laser scanning technique to estimate the fish biomass of sole populations with different individual fish size and at different stocking densities.

Senegalese sole (*Solea senegalensis*) is a flatfish of high commercial value and demand in the European market (Morais *et al*. 2014) because of its fast growth rates and high market price (Imsland *et al.* 2003).

2. Material and Methods

2.1. Rearing and fish conditions

The study was carried out in the facilities of the Escola Superior d'Agricultura de Barcelona at the Universitat Politècnica de Catalunya – BarcelonaTech. Fish were held in a raceway tank with a recirculation system. Four different fish densities of sole (*Solea senegalensis)* were tested: Very Low (VL), Low (L) Medium (M) and High (H) (Table 1). Two different sizes of sole were used and kept in two different tanks. For VL fish density the individual weight was 3.0 g ± 1.1 (mean ± SD) and they were kept in a raceway tank measuring 16 cm wide, 100 cm long and 5 cm of water depth. For L, M, and H densities the fish had 234.0 g ± 84.6 (mean ± SD) of individual weight. The standard weighing of fish was carried out by removing the fish from the water and anesthetizing them (2-phenoxyetanol solution, 0.4 ml L^{-1}) to obtain their individual weight.

In these trials (L, M and H) the fish were kept in a raceway tank measuring 40 cm wide, 310 cm long and 10 cm water depth. To get the different rearing fish density, the number of fish and the rearing area for each treatment were adjusted. The size of the rearing area was limited avoiding the access of the fish to some part of the tank. The use of a net was discarded for this objective because it would have distorted the reflection of the laser in the area adjacent to the net, since the laser beam is projected at an angle. Different materials were tested to check the level of rejection by the soles. Finally the artificial grass was used because it prevented fish from staying in the area.

The dimensions of the tank, the number of fish, their individual weight, and the resulting stocking density are summarized in Table 1.

Fish were fed daily around 1% BW with a commercial pelleted diet for soles (Skretting Gemma Diamond 1.5 for smaller and Skretting Elite Le-7 for bigger fish). The water temperature was

22.5 ^{o}C ± 0.9; dissolved oxygen 8.0 mg L^{-1} (109.5 % saturation ± 12.4); pH 7.34 ± 0.1; and salinity 38 g L^{-1}.

2.2. Laser scanning measures

2.2.1.Image acquisition

A laser lighting scanning technique described by Almansa *et al.* (2012), with some modifications, was used to measure the fish layer volume of *Solea senegalensis*. On the ceiling on top of the tank, a system of mobile rails was set. A digital camera (Nikon Coolpix P6000, 4224 x 3168 pixel of image resolution) and a laser light device (Lasiris SNF 20 mW, with wavelength of 440–710 nm) were fixed in opposite directions with an inclination of 45 degrees (Figure 1). This system of rails allowed moving both devices, laser and digital camera, at a time, keeping the same distance and inclination, without any contact with the fish or the tank that could disturb the population of soles.

A line drawn over the fish by the reflection of a flat beam of laser was captured by the digital camera. A sequence of images was taken for each experimental run with a 10 cm distance between images in L, M and H fish densities (with larger fish size 234.0 g ± 84.6) and 4 cm distance between images in VL fish density for a better adjustment to the small fish size (3.0 g ± 1.1). For each treatment four scans per day were performed during three days, obtaining a total of 12 replicates per treatment. In the VL density, the four scans were repeated during five days considering the expected greater mistake, and so obtaining 20 replicates. The same process was repeated with the empty tank.

The laser scanning samplings were done in dark conditions to enhance the laser light.

2.2.2.Image processing

The images of the reflection of laser line, both over the fish and over the empty tank, were captured with the digital camera (Figure 2b and 2a, respectively). Image analysis software was

used to analyze the images and convert the laser line into XY coordinates (Figure 2c). Both series of XY coordinates, with and without fish, were compared.

The coordinates of the bottom with an empty tank were fitted to a second order polynomial for each section of the tank. To be able to set the comparison and adjust the polynomial of the empty tank bottom with the reflection of laser over the fish layer, the reflection on the upper edge of the tank was used (A and B points in Figure 2a and 2b). The difference between the height of the laser line reflected on fish (h_1) and the height of the tank bottom (h_o) indicates the thickness of the layer of fish at each point.

The same process is repeated for each 4 or 10 cm section, depending on the treatment, along the entire tank. To convert the fish height in pixels into centimeters, the laser line over three references cubes with a known size (2 cm x 2 cm) was captured with the digital camera. The relationship between pixels and centimeters was obtained. The mean fish layer height for each section was converted into volume with the tank width (16 or 40 cm) and the distance between sections (4 or 10 cm). It must be taken into consideration that the fish layer volume will be constituted by the fish biomass and the interstitial water existing among them.

Finally, when a swimming fish was detected or when water surface was not calm due to the fish activity, the image was eliminated and repeated when the distortive situation disappeared.

2.3. Data analysis

To analyze the suitability and reliability of the laser scanning method, two different analyses were made: (1) The ratio of Fish Biomass versus Fish Layer Volume (FB/FLV) was studied; this relationship between biomass of fish and the estimated fish layer volume was established experimentally and (2) the repeatability of the methodology was assessed by comparing the

treatments and analyzing the Coefficient of Variation (CV) of the estimated fish layer volume in the different replicates for each treatment.

2.3.1.Ratio of Fish Biomass versus Fish Layer Volume (FB/FLV)

In order to compare the sampling methods for each treatment (VL, L, M and H), the ratio FB/FLV was obtained for each one. The relationship between the fish biomass and the fish layer volume was calculated using the biometry of 100 % of the sole population as reference and assuming that fish and water densities were equal. Values for the ratio FB/FLV closest to 1 mean that fish layer volume are almost totally occupied by fish biomass. Instead, values above 1 denote the presence of interstitial water occupying a fraction of the measured fish layer volume.

Normality of the data with Shapiro-Wilk test and homogeneity of variance using Barlett's test were tested to ensure the assumptions for analysis of variance were satisfied. A Person's correlation of volume layer measured with laser scanning method with the total fish biomass was performed. The ratio FB/FLV for different stocking densities was evaluated by an ANOVA test using the SPSS software. When differences were found, a Tukey test was used to evaluate them. In all cases a level of 0.05 was taken as significant.

2.3.2.Repeatability of measurements

To evaluate the feasibility of the method, we calculated the Coefficient of Variation (CV) of fish layer volume measured for the 12 replicates of the L, M and H experiments; and for the 20 replicates of VL experiment.

3. Results

In order to analyze the reliability and accuracy of laser scanning method in different situations, it was tested with four different fish densities: VL, L, M and H and two different fish sizes. The results are analyzed below.

3.1. Ratio FB/FLV

Positive and significant correlation was obtained between the fish layer volume measured by laser scanning method and total fish biomass measured by a biometry of 100% of the sole population.

The relationship FB/FLV was always lower than 1. This could be due to the presence of interstitial spaces occupied by water that contribute to increase the volume of fish layer. The percentage of this interstitial water volume could be estimated by:

$$\%\ Interstitial\ water\ volume = \left(1 - \frac{FB}{FLV}\right) \times 100 \qquad \text{(Equation 1)}$$

When the relationship between ratio FB/FLV and stocking density is considered, the value of the ratio is clearly lower in VL treatment (0.4 Kg m^{-2}) than in the others. It increases markedly between the VL and the L treatment (Figure 3).

Contrarily, the increase from M to H treatment is more attenuate. For this reason it can be guessed that although the fish density increases, the relationship between the ratio FB/FLV and stocking density will be similar.

There were no statistical differences ($p > 0.05$) between the ratio FB/FLV obtained with the M and H stocking densities and larger fish. In both treatments the ratio was higher than 0.95 (0.957 ± 0.021 and 0.967 ± 0.011 respectively). It means that interstitial water estimated by equation (Eq.1) represents less than 5% (Table 2). In the L treatment, the ratio decreased to 0.914(± 0.034) and so the estimated interstitial water volume increased to 8.6 %. Nevertheless,

in VL fish density and with smaller fish, the ratio was much lower (0.752 ± 0.054), giving an estimation of the interstitial water volume close to 25 %.

The increase in this percentage when the fish size and stocking density decreases to extremely low values (3.0 ± 1.1 g and 0.4 kg/m^2) and there is not fish overlapping in the fish layer, does not seem very realistic. Taking into account that the stocking density 0.4 kg/m^2 would correspond to an average thickness of the fish layer of 0.4 mm, it seems more reasonable to assume that the smaller value of ratio FB/FLV observed with the smallest stocking density was due to the resolution of the image catching process, which needs to compare the laser line projected on the fish layer with the laser line previously projected on the bottom of the empty tank. A small error around 0.1 mm in the reference level of the empty tank would produce an error around 0.1 kg/m2 in the determination of the stocking density. This represents a very small percentage when working with medium and high stocking densities, but is very relevant in the smallest density.

3.2. Comparison of treatments

When the comparison of treatments is considered, the coefficients of variation (CV) calculated in all fish densities tested were very low, with a maximum value below 7.5 % (Figure 4). The CV ranged from 7.2 % in VL stocking density to 1.1 % in H density. A variation of CV was observed in terms of fish density, with a tendency to decrease as the stocking density increases. The tendency is more pronounced in VL and L trials. Between L and M density, the difference in CV in relation to the density of fish was greater than that observed between M and H trials, which were very low. According to the results, it seems that beyond a certain stocking density, a change in this parameter does not lead to a proportional change in CV, which remains quite constant (Figure 4).

These results, together with the Ratio FB/FLV, lead to the assumption that differences in CV, which are greater in VL treatment, are mainly due to the fish size.

4. Discussion

The results obtained to estimate flatfish volume with laser scanning show that this method has a great potential as a basis to develop an efficient feed and stock management without stress, and provides a biomass estimation of the complete population very easily.

The measure of the total fish biomass in aquaculture facilities is of great importance, but the technique more currently used (extracting fish out of water and weighing them) is intense labor and causes great stress of fish (Ashley 2007). For this reason, for the last three decades diverse alternatives have been investigated. With this purpose, the laser scanning technique to measure the total flatfish biomass exposed here, eliminates the need to anesthetize or handling the fish.

On the other hand, the morphological and habitat characteristics of flatfish lead to rule out other sampling systems by some of the previously studied image analysis (Ruff et al., 1995; Bedow et al, 1996; Sheih and Petrell, 1998; Tillet et al., 2000; Lines et al., 2001; Martinez-de Dios et al., 2003; Costa et al., 2006, 2009; Hufschmied et al., 2011). Particularly, they make easier their study in 2D through the volume occupied by the layer of fish in the tank bottom. In this case, the main drawback which is found with flatfish is the fact that the layer of fish is not formed only by biomass, but also includes the water remaining among them which leads to an overestimation of the actual volume of fish (Almansa et al. 2012). Alike, Storberck and Daan (1991) found an error measuring dead flatfish over a belt by laser light and image analysis when the fish was suspended above the belt due to rigor mortis; in this case, the underlying air volume was then seen as part of the volume.

The overestimation of the volume of fish found in this study is related to the fish size, thus the smaller the fish, the ratio of FB/FLV was farthest to 1. With higher fish (234.0 g ± 84.6) and higher rearing densities, the overestimation caused by the presence of interstitial water was between 3.3 and 4.3 % with no significant differences. These values were lower than those

previously obtained by Almansa et al (2012) (between 11 – 15 %) presumably because the characteristics of the mobile device used and the tank structure which were used then, did not allow images to be taken in both ends of the unit (inlet and outlet) and the fish density in those areas was estimated from the measured density in the closest area.

In the results presented here, the lowest value of ratio FB/FLV obtained when working with the smallest fish (3.0 g ± 1.1) at the lowest density, may be attributed to the resolution of the image catching process which occurs in this extreme scenario. It indicates that this laser scanning technique would not be feasible for the size of flatfish found in hatcheries, while it can be very useful for estimating the flatfish biomass in on-growing facilities. Even though, the values of CV obtained with the worst scenario, very small fish and the lowest stocking density, were really low (between 1.1 -7.2 %), and allows classing the method, in all conditions tested, as reliable and accurate. Especially with standard conditions of sole culture, as far as fish size and stocking density are concerned, which are usually between 70-100 g and 300-350 g individual fish weight, and between 10-12 Kg m^{-2} and 22-25 Kg m^{-2} of stocking density (Rodríguez and Peleteiro, 2014) (here, with 22.8 Kg m^{-2} and individual weight of 234 g ± 84.6).

Some other features of the method should be highlighted. First, the method is not dependent on a specific behavior of the fish; it does not require that animals do anything special. The systems described by Hufschmied et al. (2011) or that developed by Vaki Aquaculture Systems Ltd (VAKI 2009), need the fish to pass through a particular device. Some fish species, such as flatfish, are reluctant to go through these devices, which can increase their level of stress. Furthermore, measurements of the fish with these systems could not be representative of the whole fish population (Martinez-de Dios et al., 2003). Second, these methodologies are designed to measure the “round fish” biomass and so they require the view of a whole fish (Ruff et al., 1995; Bedow et al., 1996; Israeli and Kimmel, 1996; Stien et al., 2007; Costa et al., 2009; Hufschimied et al., 2011). Fish overlapping is the most common circumstance in flatfish

culture, so it wouldn't be possible to use them with these species. Moreover, as reported by Bedow et al. (1996), accuracy of the methods decreases with decreasing fish size and is strongly dependent on the distance from the fish to the camera.

Finally, the usability of this system allows the procedure to be repeated regularly and frequently, since it avoids the stress produced by fish handling so reducing the negative consequences on growth and mortality (Flos et al., 1988; Barnett and Pankhurst, 1998). The device also have other utilities, as demonstrated in Almansa et al. (2012), with a special emphasis on its feasibility as a tool to study flatfish distribution in a raceway and so detect modifications in response to changes in environmental conditions while biomass measurements obtained are reported.

It's important to point out, though, that the objective of the method is to sample the fish biomass, it is not an individual sampling method, so it does not give information on the distribution of fish size of the population. Therefore, it would still need to manually sort the fish or the use of automatic sorting.

Otherwise, the automatic image analysis and the laser scanner share a limitation related to the illumination irregularities or the presence of unwanted objects, such as bubbles (Martinez-de Dios et al., 2003). All of them may be affected by this problem, the difference is that laser scanning allows a rapid detection of the problem, making easy to discard the affected image and repeat the operation. In an automated system, the criteria to discard the images where an unwanted object or a swimming fish is intercepted by the laser beam in the water column should be based in the discontinuity of the line drawn by the laser beam and the presence of a line portion located high above the main line.

All these features mentioned above make the laser scanning technique a valuable tool to estimate the total fish biomass in growing flatfish tanks. Its automation would allow the

periodic measurement of the total biomass non-invasively to easily establish, among others, the feeding regime, the antibiotic dose or to calculate the oxygen consumption.

5. Conclusions

This technology has the potential to accurately monitor flatfish biomass in commercial growing facilities with high stocking density.

The overestimation of fish volume obtained by laser scanning method with stocking densities between 12 and 23 Kg m^{-2} is mainly due to the interstitial water volume present among fish.

With fish around 3 g and stocking densities below 6 Kg m^{-2} the overestimation of the method is mainly related to the difficulty of establishing the actual bottom of the tank in laser image on the layer of fish. In this case the percentage that represents a pixel is greater than in higher rearing densities.

The lower coefficients of variations obtained allow classing the method as reliable and accurate to estimate flatfish without relying on fish size.

The proposed method could be an alternative to the common biometry method of subsample weighing by reducing the labor involved and the stress commonly associated to manual sampling.

Acknowledgements

This work was funded by Spanish Ministerio de Educación y Ciencia (AGL2009-1655).

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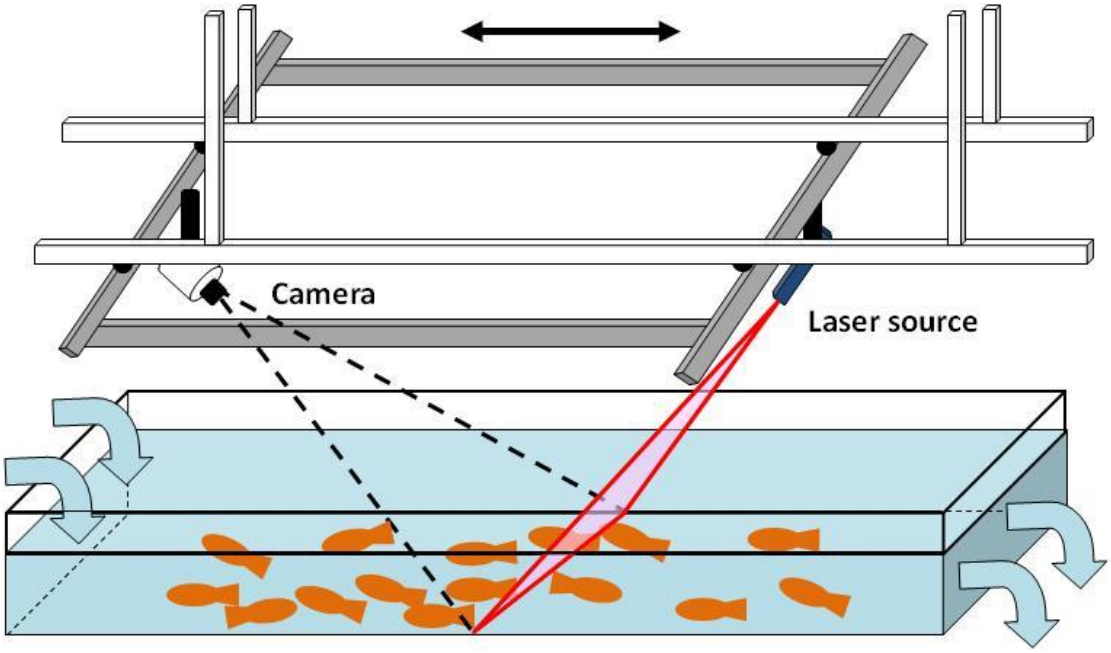

Figure 1: Schematic drawing of the mobile rails over the tank with the camera and laser attached

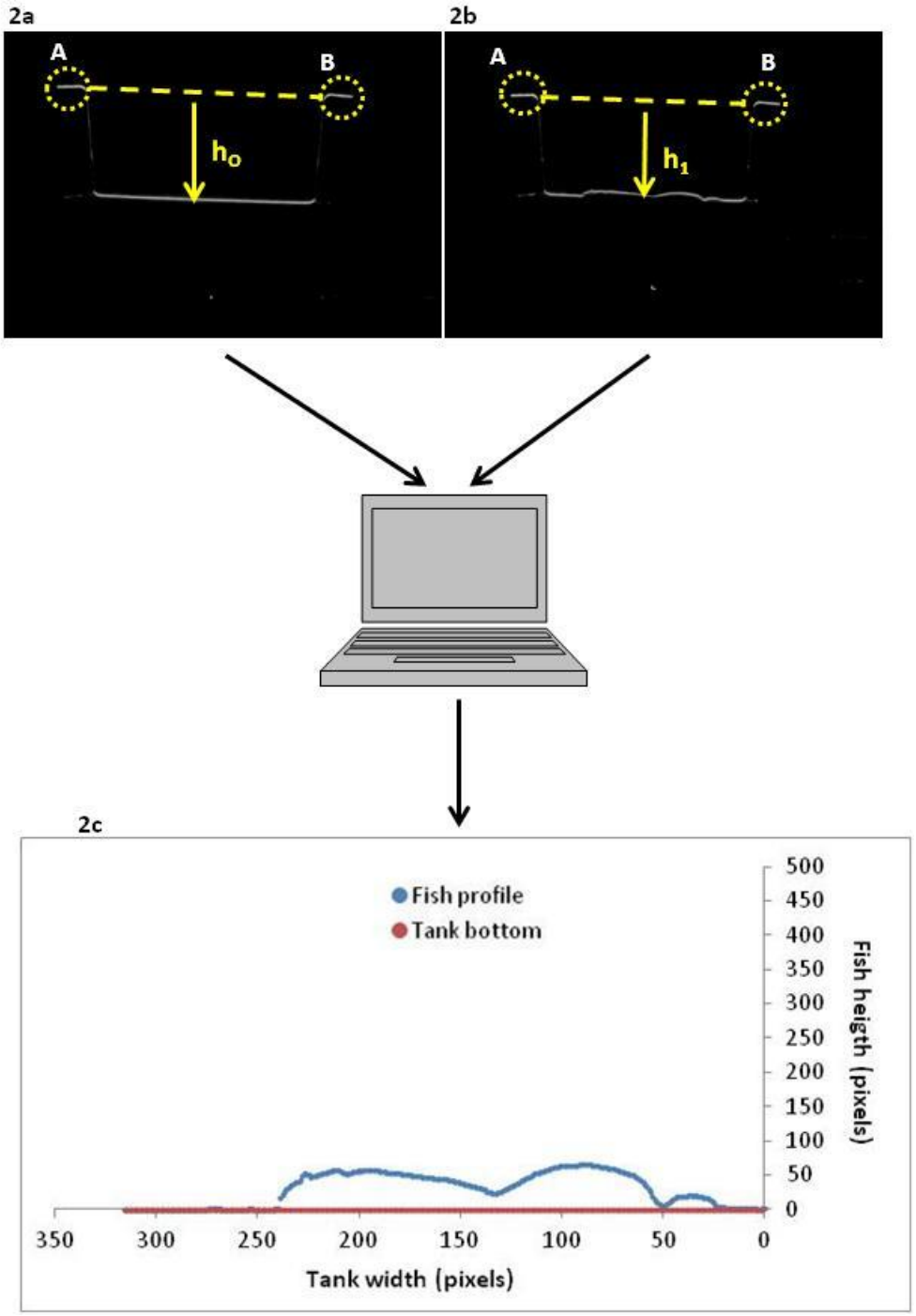

Figure 2: (2a) Reflection of laser line in the tank without fish. **(2b)** Reflection of laser line, in the same tank, with fish. The circled objects (A and B) are the laser reflections over the upper edge of the tank used to adjust each pair of images. **(2c)** Fish profile on the cross section taken from image shown in **2b**.

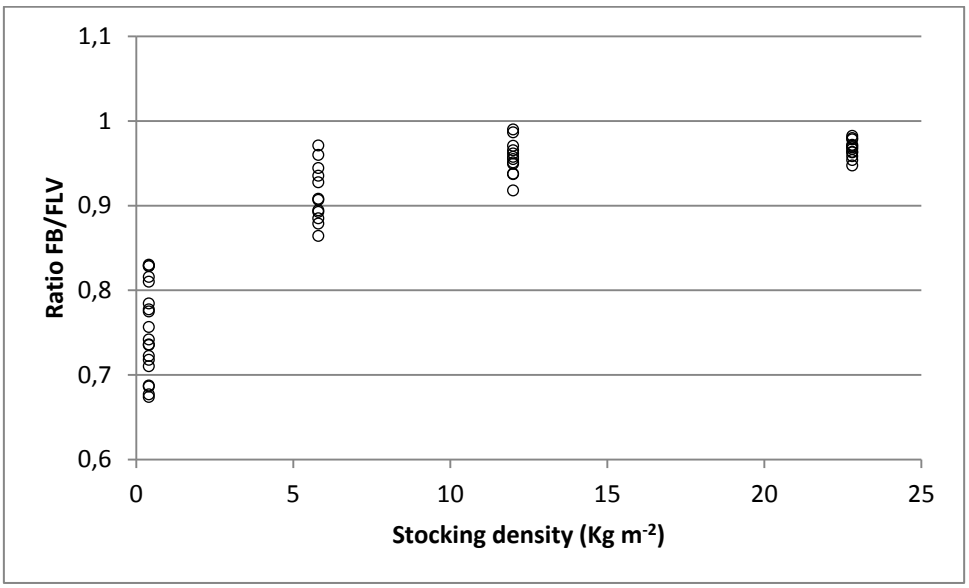

Figure 3: Relationship between ratio FB/FLV and stocking density (Kg m^{-2}). N = 20 in VL (0.4 Kg m^{-2}) and N = 12 in the L (5.8 Kg m^{-2}), M (12.0 Kg m^{-2}) and H (22.8 Kg m^{-2}).

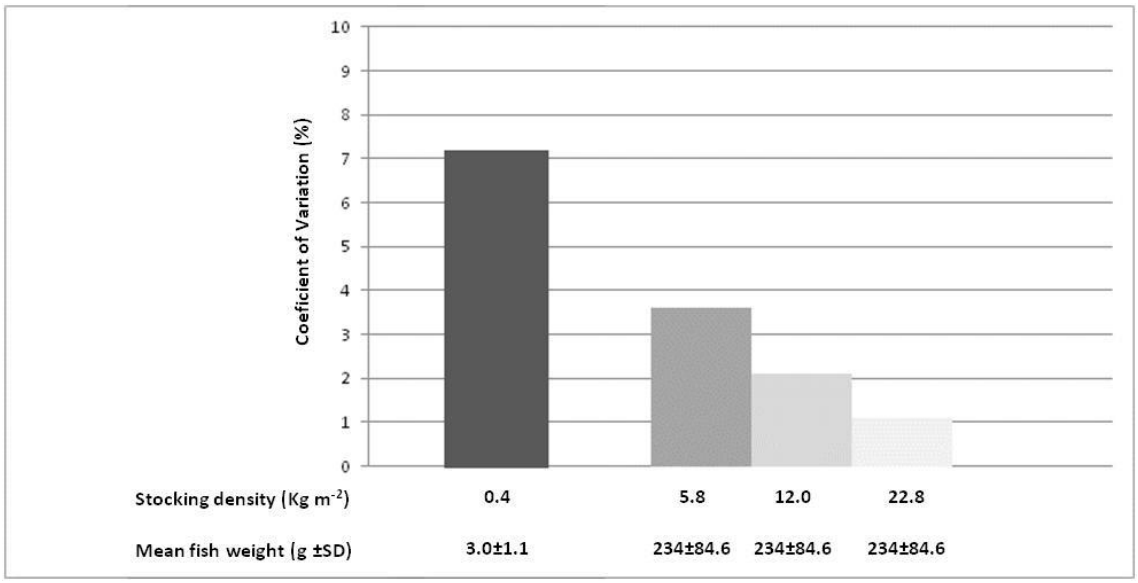

Figure 4: Relationship between stocking density (Kg m^{-2}) and Coefficient of Variation (CV)of the fish layer volume measured for the replicates.

Table 1: Stocking conditions

Qualitative fish density	**Tank dimensions (cm x cm)**	**Number of fish**	**Mean fish weight ± SD (g)**	**Stocking density (Kg m^{-2})**
Very Low (VL)	100 x 16	22	3.0 ± 1.1	0.4
Low (L)	270 x 40	27	234.0 ± 84.6	5.8
Medium (M)	190 x 40	39	234.0 ± 84.6	12.0
High (H)	100 x 40	39	234.0 ± 84.6	22.8

Table 2: Results of total fish biomass and fish layer volume obtained with different methods and the 95 % confidence interval of the mean of fish layer volume obtained by laser scanning method, the ratio FB/FLV (± SD) and the interstitial water volume (± SD).

Qualitative fish density	Total biomass (g) by standard sampling	Fish layer volume (ml) ± SD by laser scanning method	95 % Confidence interval (lower – upper limits)	Ratio FB/FLV	Interstitial water volume (%)
Very Low	66.3	88.5 ± 6.3	85.5 – 91.6	0.752 ± 0.054[c]	24.8 ± 5.4
Low	6317.0	6919.2 ± 252.2	6758.9 -7079.5	0.914 ± 0.034[b]	8.6 ± 3.4
Medium	9124.0	9541.0 ±204.8	9410.9 – 9671.0	0.957 ± 0.021[a]	4.3 ± 2.0
High	9124.0	9435.7 ± 103.3	9370.1 – 9501.4	0.967 ± 0.011[a]	3.3 ± 1.1